Quantitative Simulation on Concentration-Time Profiles of Oxycodone Co-administration with Diazepam

by

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Abstract

In recent years, drug abuse has posed a great threat to public health. Among abused drugs, prescription opioids have caused a significant problem. Oxycodone is a pervasive semi-synthetic prescription opioid, indicated for treatment of moderate to severe pain. Marked as a Schedule II controlled substance, it possesses a high potential for abuse. Along with Hydrocodone and Methadone, Oxycodone has become one of the three most commonly overdose-involved prescription opioids. Previous studies indicate that the combination of oxycodone and benzodiazepines can render higher overdose liability and stronger effects. Other researchers have examined their pharmacological interactions by experiments and explained this problem's severity from statistical angle. As both drugs undergo phase I metabolism through CYP 3A4 enzyme, we believe that their concurrent use produces drug-drug interactions (DDIs), leading to increased plasma concentration and prolonged CNS effects. The goal of my research is to quantitatively simulate the concentration-time profiles of oxycodone co-administered with diazepam (a type of benzodiazepines). First, we performed

statistical analysis of 40,996 records in FDA Adverse Event Reporting System (FAERS) for oxycodone. The result shows that most death outcomes involve concomitant use of oxycodone with other drugs, and that about half of these co-administrations include benzodiazepines, which manifests the significance of this problem. Based on the pharmacokinetic data of oxycodone administered in different doses, we built a one-compartment model with first-order dosing and linear elimination. Using the model, we obtained the parameters of single oxycodone pharmacokinetic simulations. With the foundation of the model and parameters, we simulated the oxycodone concentration-time profile in the case of concomitant use with diazepam and drew the conclusion. While co-administered with Diazepam, the metabolism of Oxycodone is delayed and renders more threatening overdose symptoms. By conducting the quantitative simulations on pharmacokinetic profiles of oxycodone and its high-risk DDIs, we explored the potential threshold of this overdose and provided rational drug use instructions.

Key words: Drug Abuse; Co-administration; Oxycodone; Diazepam; ODE Model; Compartment models; Quantitative Simulation

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PREFACE

I sincerely appreciate my advisor, Dr. Xiang-Qun Xie, whose instructions and support from the beginning to end encouraged me to start, proceed and finish the project. The project he assigned me has improved myself a lot.

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I would like to thank my committee members, Dr. Xiang-Qun Xie, Dr. Levent Kirisci, and Dr. Lirong Wang. Their precious advice enlightened me and assisted me to complete my thesis.

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I would like to show my gratuity to all the members in Dr. Xiang-Qun Xie's group, who accompanied me and helped me a lot in the last two years.

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Lastly, I offer my regards and blessings to all of those who supported me in any respect during the completion of the project.

1.0 INTRODUCTION

1.1 DRUG ABUSE

Drug abuse has posed serious issues to public health. Drugs with abuse liability can be grouped as stimulants, analgesics & narcotics, hypnotics as well as antidepressants, nicotine and alcohol [1]. In this section, we would like to focus on the abuse study of prescription opioid, a class of analgesics & narcotics. Over the past 15 years, the use of opioid pain killers in the United States has soared [2]. In 2012, epidemiologic data indicate that 12.5 million Americans reported the abuse of prescription opioids [3]. During 2014, 28647 drug overdose deaths involve opioids [4]. The majority of overdose deaths are caused by concomitant use of multiple prescribed or illicit substances, with the most common factor, benzodiazepines. Previous data have supported this fact. In 2008, 62% of the emergency department visits for narcotic analgesics involved multiple drugs and among them, including alcohol, 26% involved benzodiazepines use [5]. Benzodiazepines are also pervasively abused drugs. In 2011, 426000 emergency department visits in the US involves nonmedical use of benzodiazepines [6].

People have come up with several ways to solve the drug abuse issues, like policy alteration, physician training, patient education and monitoring programs. However, none of these solutions seems effective. Numerous of problems appear in the process. The treatment of prescription opioid addiction has long been neglected. Neither

pharmacotherapy nor behavioral treatment presents sufficient efficacy. The prescription drug monitoring programs are hard to conduct as the difficulty to find the data [7].What makes the problem more complex is the use of prescription opioids all depends on the patients themselves. In the view of experts, the recovery is likely best achieved through a combination of pharmacotherapy and counselling [8].

Given the dilemma of drug abuse investigation, my research project proposes to conduct the quantitative simulation of a prescription opioid, oxycodone and its co-administration with a benzodiazepine, diazepam. By exploring the potential threshold of overdose, we hope to better assess the risk of toxicity, provide the rational drug use instructions and help the decision making.

1.2 OXYCODONE

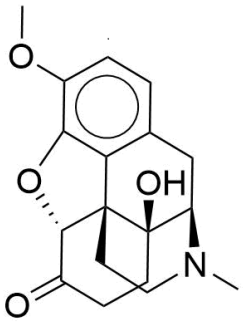
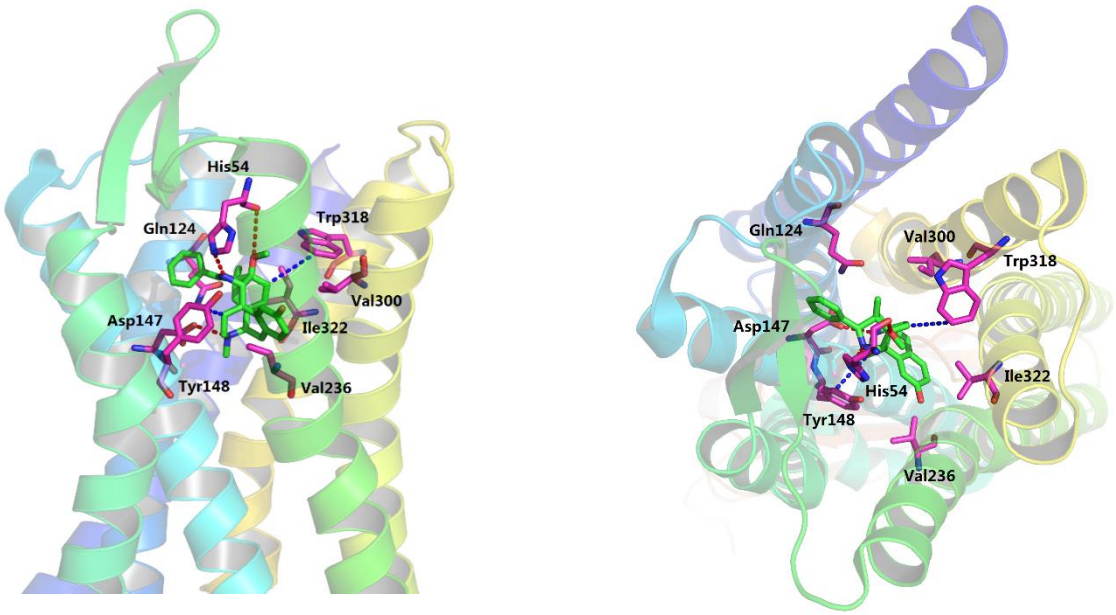


Figure1. Chemical structure of Oxycodone

Oxycodone, (14-hydroxy-7, 8-dihydrocodeinone, $C_{18}H_{21}NO_4$) [9], is a semi-synthetic opioid [10] and pervasive narcotic analgesic, indicated for the treatment of moderate to severe pain. Oxycodone can be administered orally, rectally, intraspinally and

parentally [11]. In my research, I choose the controlled-release oral formulation of oxycodone to study. This kind of oxycodone is sold under the brand name OxyContin. The oral bioavailability is about 60%-87% [10]. As a controlled-release drug, OxyContin has a two-phase release-absorption process, with two half-lives, 0.6h and 7h and its plasma half-life is between 3-5 h [10].The dosage forms include 10, 15, 20, 30, 40, 60,80 mg film-coated tablets while the dosing regimen varies individually.



(a) (b)

Figure 2. Crystal structure of active mu-opioid receptor bound to agonist BU72 (PDB ID: 5C1M)

(a) Membrane view (b) Extracellular view

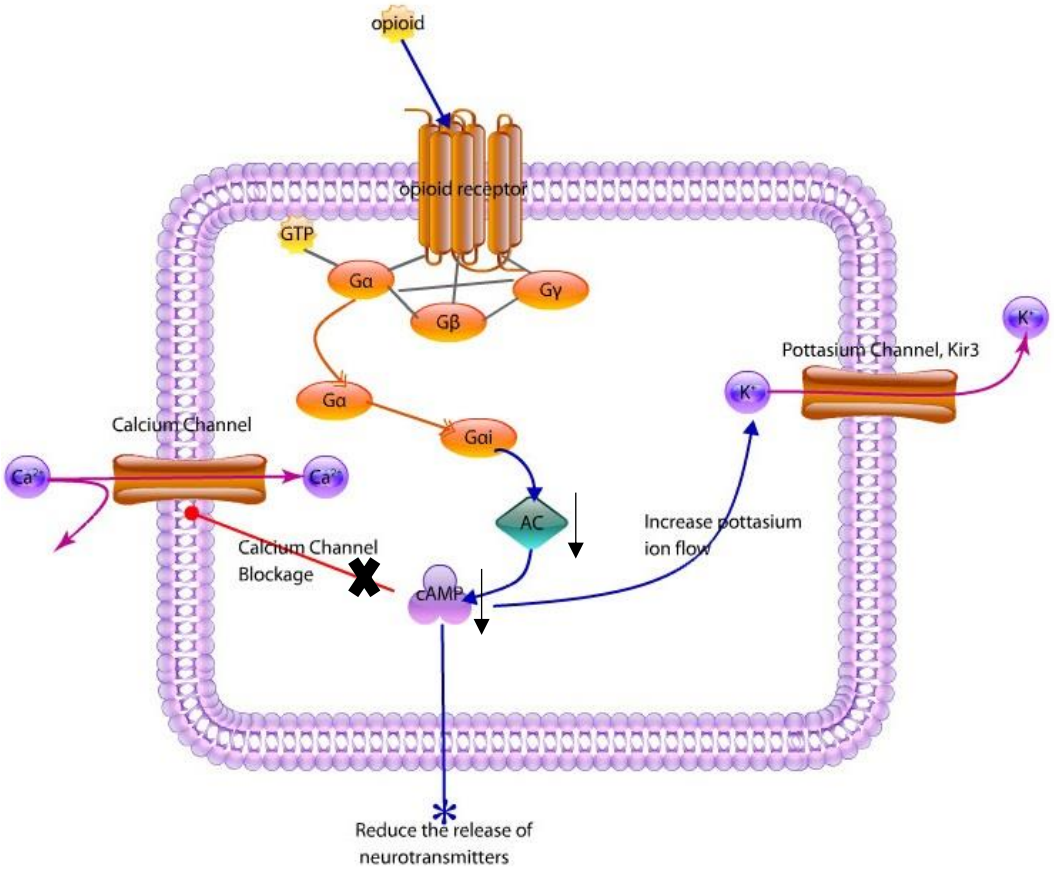


Figure 3. Oxycodone mechanism of action

Oxycodone achieves its pharmacological effect through binding to opioid receptors located in central, peripheral and autonomous nervous systems. Opioid receptors are seven-transmembrane G-protein-coupled receptors (GPCR). By coupling to G-proteins, these receptors trigger a cascade of intracellular activities. The Gα subunit interacts with potassium channel, Kir3, rendering cellular hyperpolarization and tonic neural activity inhibition [12]. Besides this, opioid receptors cause Ca2+ influx reduction and adenylate cyclase inhibition. Finally, these activities inhibit spinal cord pain transduction and reach the analgesic effect.

Oxycodone is marked as a Schedule II controlled substance which means it has a high potential for abuse. There are specific terms in OxyContin label about drug overdose which is primarily manifested by respiratory depression. The occupancy of opioid receptors renders reduced ventilator frequency and pattern. Such activity profoundly depresses the HVR (hypoxic ventilator response) and HCVR (hypercapnic ventilator response). Previous investigations on rats show that pre-Bötzinger complex and retro-trapezoid and parafacial respiratory group (RTN/pFRG) play a vital role in respiratory depression. The pre-Bötzinger complex and the RTN/pFRG are small areas in the brainstem that can generate respiratory rhythm [13]. The pre-Bötzinger complex is active during expiration and the pre-Bötzinger complex is active during inspiration. However, the pre-Bötzinger complex can be inhibited by opioids while the pre-Bötzinger complex is not affected. As a result, this different sensitivity to opioids leads to an irregular respiratory rhythm. Although this theory hasn't been verified in humans, it provides a possible explanation for opioid-induced respiratory depression.

1.3 DIAZEPAM

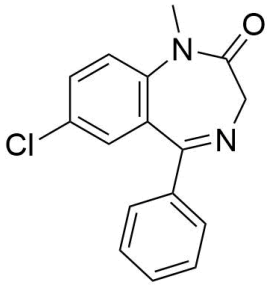


Figure 4. Chemical structure of Diazepam

Diazepam(DZP),7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one [14], $C_{16}H_{13}CLN_2O$) , a type of benzodiazepines, was launched to the market in 1963 [15]. With the brand name, Valium, it is indicated for anxiety disorders, epilepsy, muscle spasms, and alcohol withdrawal. The bioavailability ranges from 93 to 100% orally [16] which is relatively high. When delivered orally, diazepam will reach the peak plasma level (C_{max}) pretty soon, just 30-60 min after administration [16]. However, as the diazepam metabolites are active, the half-life can be as long as 24 to 48 h [17]. The initial dosage regimen for adults is between 2 to 10 mg orally, 2-4times daily.

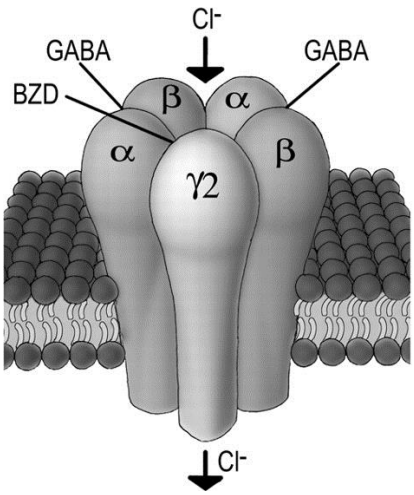


Figure 5. GABA receptors [18]

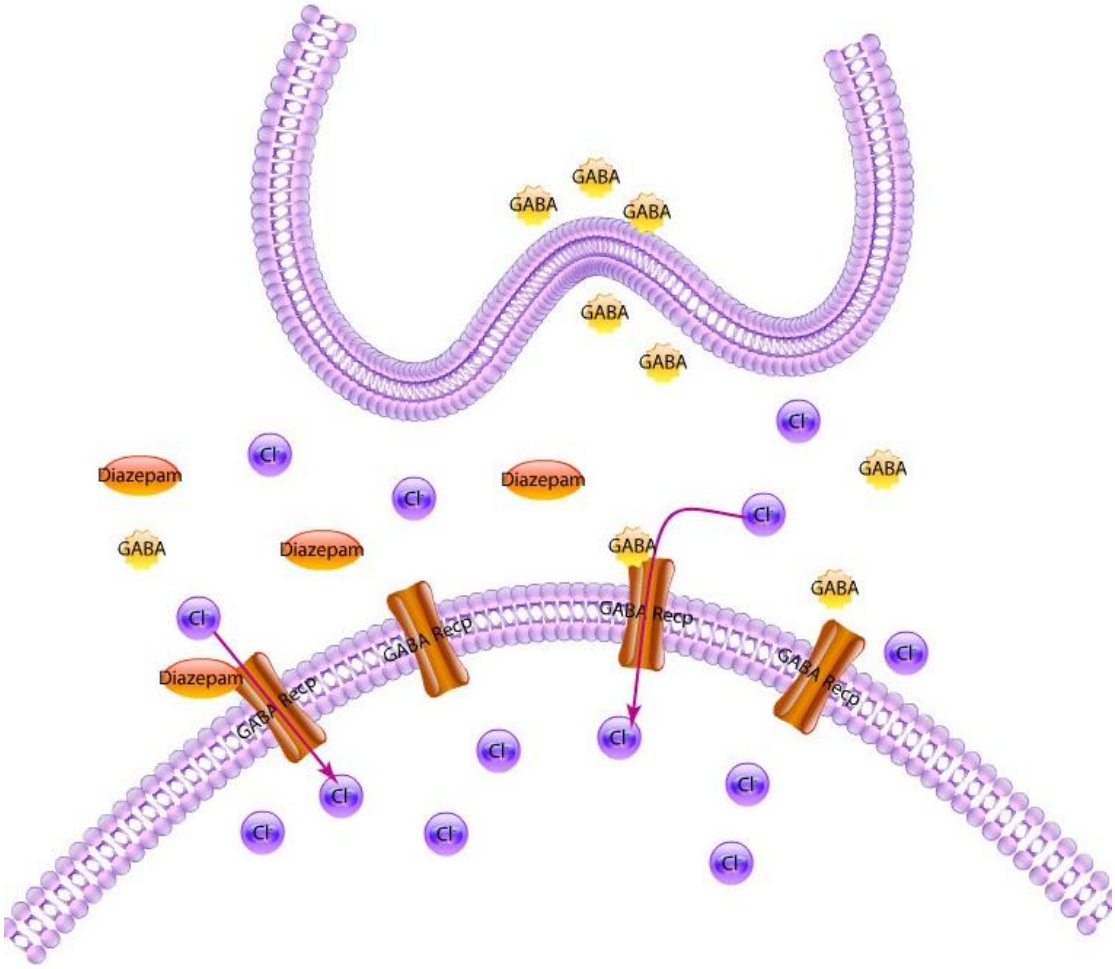


Figure 6. Diazepam mechanism of action

In terms of its high lipophilicity [19] and protein binding rate, diazepam has a rapid onset [20] and can easily cross the blood-brain barrier which means it possesses central nervous system (CNS) effect. Diazepam can be regarded as a positive allosteric modulator of $GABA_A$ receptors [21]. This receptor is a ligand-gated chloride-selective ion channel, composed of 5 glycoprotein subunits, 2α, 2 β and 1γ

subunit [22] . By binding to a site far from GABA binding site, diazepam causes the conformational change and activates $GABA_A$ receptors. This gives rise to increased chloride conductance as well as the frequency of channel openings [23]. As GABA is the most common neurotransmitter in CNS and has an inhibitory nature, such activity significantly reduces the excitability of neurons [22].

The typical side effects of diazepam are manifested by psychomotor retardation, memory impairment, paradoxical disinhibition, depression and emotional blunting [24]. Diazepam is marked as schedule IV controlled substance [25]which means it also possesses abuse potential. Under the FDA instructions, diazepam should be used very cautiously in patients with drug abuse history. There is potential drug-drug interactions (DDIs) existing. The overdose symptoms of benzodiazepines are CNS depression, ranging from drowsiness to coma.

1.4 CO-ADMINISTRATION MECHANISM

Both Oxycodone and diazepam are mainly metabolized in the liver. After oral administration, oxycodone undergoes phase I metabolism via CYP3A4 and CYP2D6. Generally, 45% of oxycodone is N-demethylated to Noroxycodone by CYP3A4 and another 11% is O-demethylated to Oxymorphone through CYP2D6 [26]. The main metabolite Noroxycodone is relatively inactive while Oxymorphone is active [27]. In this way, it can be seen that the parent drug produces pharmacological effects. The metabolic pathway is shown as follows.

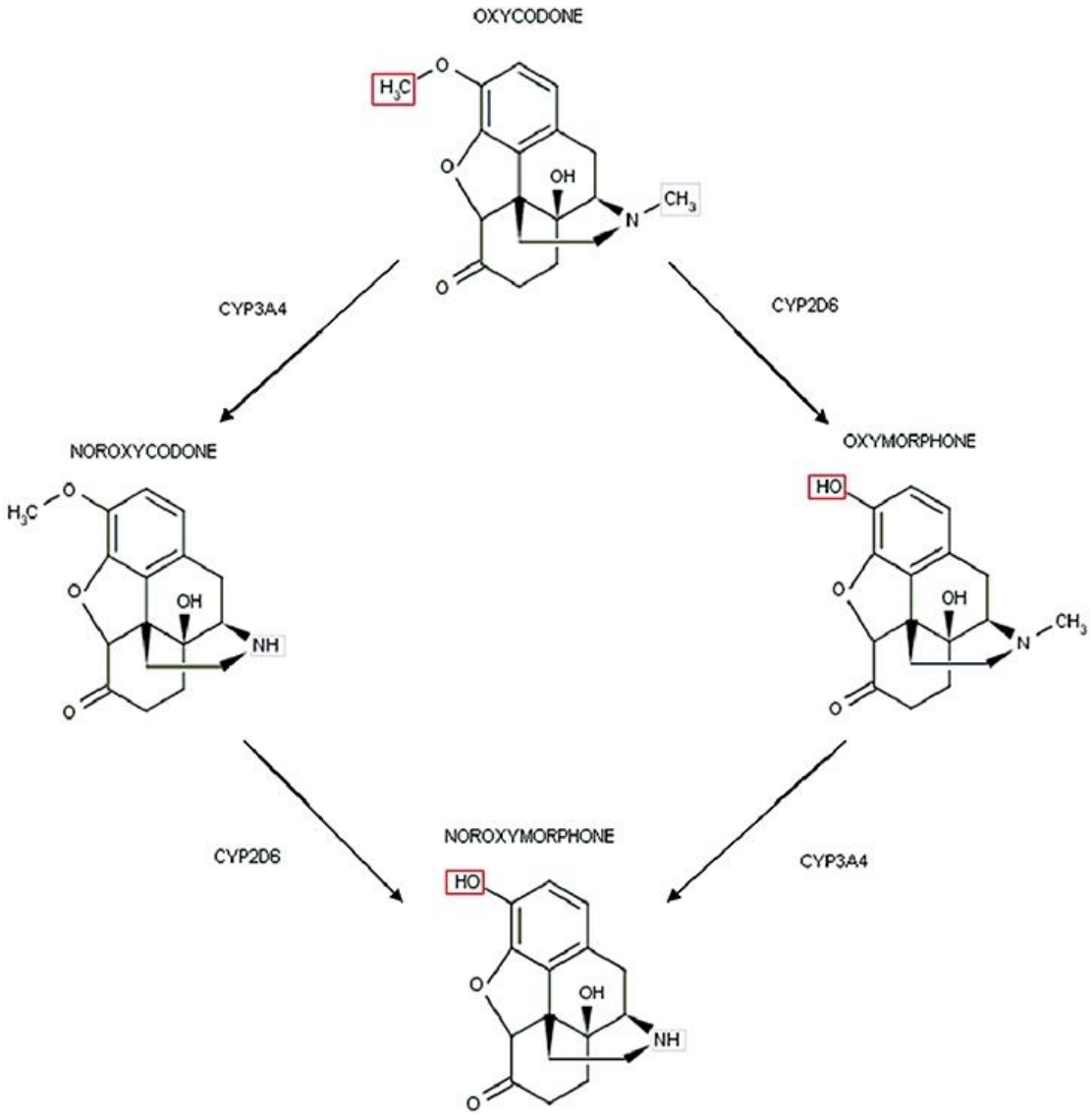


Figure 7. Oxycodone metabolic pathway[28]

The metabolism of diazepam is mediated in the liver through cytochrome P450 (CYP450) enzymes, mostly CYP3A4 and CYP2C19 [29]. Diazepam is metabolized to nordiazepam via N-demethylation and temazepam by C_3 hydroxylation [30]. Both nordazepam and temazepam will be metabolized to oxazepam [31]. The three main diazepam metabolites nordiazepam, temazepam and oxazepam are all active and have

been marketed as drugs [16]. This fact explains why diazepam has a long half-life and consistent efficacy. The metabolic pathway of diazepam is as follows.

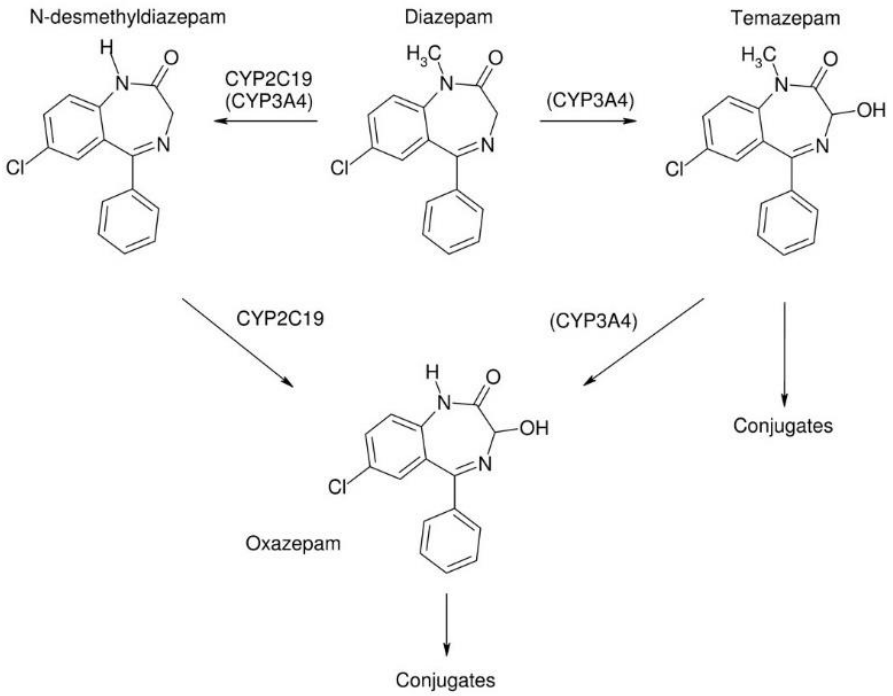


Figure 8. Diazepam metabolic pathway [18]

From the two metabolic pathway figures, we can see that both oxycodone and diazepam are metabolized by CYP3A4 enzyme. According to the data from SuperCYP, diazepam can be considered as substrate and inhibitor of CYP3A4. It is already known that both inhibitors and substrates of a particular CYP isozyme decrease the metabolism of substrates of that enzyme. As mentioned, oxycodone has neural inhibitory function as well as diazepam triggers inhibitory-natured neurotransmitter GABA. We propose that there is potential drug-drug interactions (DDIs) between these two drugs. This DDI can lead to higher plasma concentration

and prolonged CNS effects. The combination produces additive or synergistic effect, increasing the risk of acute harms such as overdose [32].

Name	2C19	2D6	2E1	3A4	3A5	3A7	3A43
Diazepam	Inh S		Ind	Inh S	S	S	
alternative drugs for Diazepam	**2C19**	**2D6**	**2E1**	**3A4**	**3A5**	**3A7**	**3A43**
Bromazepam	S		Inh	S			
Clotiazepam	S			Inh S			
Clobazam	S			S			
Etizolam	S			S			
Nordazepam	S			S			
Alprazolam				S	S	S	
Tofisopam				Inh			
Adinazolam	S			S			
Lorazepam							
Oxazepam				S	S	S	S
Chlordiazepoxide		S		S			
Prazepam				S			
Medazepam	S		Inh	S			

Figure 9. Drug-drug interaction on SuperCYP

S: Substrate, Inh: Inhibitor

1.5 CO-ADMINISTRATION CONTEXT

Certain medications, for example, analgesics, antidepressants and anxiolytics, have been identified as significant risk factors in adverse drug events [33]. The top 10 drugs involved in drug overdose deaths in the United States from 2010 to 2014 belong to 3 drug classes: opioids, benzodiazepines and stimulants [34]. Recent studies have reported a rise in oxycodone abuse and the majority of deaths attributable to

oxycodone are cases of combined drug toxicity. Oxycodone, as a commonly-used narcotic analgesic, ranks within top 3 throughout the years [34]. In the meantime, diazepam, a member of benzodiazepine family, has been listed in this rank for five years [34]. Previous researches have reported a the majority of deaths attributable to oxycodone are cases of combined drug toxicity [35]. Among all these combination cases, benzodiazepines are the most pervasive co-toxicants [35].

There are plenty of studies showing these two drugs have been used together for medical or non-medical purposes. It is widely believed that co-prescription of opioid with other medications may assist in the management of chronic pain [36]. For instance, sometimes Oxycodone and Diazepam are used concurrently in chronic nonmalignant pain (CNMP) patients on long-term opioid treatment related to anxiety or sleep disorders [37]. However, if people take prescription opioids and benzodiazepines concurrently at supratherapeutic doses in recreational settings, or take the drugs in a manner not prescribed by the physician, the degree of adverse effects, especially respiratory depression, can render serious medical consequences [38]. Such evidence emphasizes the need of better patient education, close patient monitor and thorough benefit-risk assessment when prescribing these drugs in combination [33].

2.0 METHODS

2.1 SOFTWARE

The aim of our research is to quantitatively describe the concentration-time profile of oxycodone and its co-administration with diazepam which is related to the emerging science, pharmacometrics. It is designed to conduct quantitative analysis of pharmacokinetic and pharmacodynamic data [39]. In this section, I want to introduce a few softwares usually applied to pharmacometrics study, for example, NONMEM, Matlab, R, SIMCYP Simulator, Monolix, Simulx, PFIM, PopED, win(open)BUGS [40].

NONMEM, which was introduced in 1982, is the acronym of NON-linear Mixed Effects Modeling [41]. Just as the name implies, NONMEM has been significantly applicable to population PK/PD studies. This software considers the nonlinear regression models which are capable of estimating population PK/PD parameters [42]. The latest version of NONMEM is 7.3.0 with Monte Carlo expectation-maximization and Markov Chain Monte Carlo Bayesian methods added in 2013.

R is a programming language and software environment for statistical computation and graphics [43]. It contains linear and nonlinear modeling, statistical tests, time-series analysis, clustering, classification and so on. An R package called RxODE to solve ordinary differential equations (ODEs) and perform simulations in pharmacometric models [44]. The latest R 3.3.3 will be released on March 6, 2017.

SIMCYP is a platform and database for bottom-up mechanistic modeling and

absorption, distribution, metabolism and excretion (ADME) simulation [45]. It takes into account the factors like experimental data, dosage forms, drug properties and population variances. SIMCYP is capable of handling enormous physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) models. In recent years, this software has evolved from a simple DDI tool to a sophisticated and comprehensive model based drug development (MBDD) platform that covers the whole drug development process [46]. Now it's been developed to version 16.

Matlab, abbreviation of matrix laboratory, is a multi-paradigm numerical computing environment. I will focus on the introduction of SimBiology, the Matlab package I utilized in my research. According to the SimBiology tutorial, it is a versatile tool to model, simulate and analyze dynamic systems, especially focusing on PK/PD and system biology studies. The operation procedure of SimBiology is as add data—add model—add task. In this way, first users add experimental or any sort of data, then build the model. These models can be imported from the built-in PK libraries or created individually. It's also feasible to integrate the inset PK models with customized system biology models. In the model session, we can set various parameters. The last step will be "Add task" where a number of commands can help users implement analysis. For instance, the 'Fit data' option can be applied to population pharmacokinetics. Generally, the simulation is based on ODEs. However, if none of the commands can meet the researchers' needs, then they can use 'create custom analysis' to generate their own codes. A great many pharmaceutical researches can be achieved by SimBiology, such as population pharmacokinetic modeling in

using nonlinear mixed-effects methods, using system pharmacology modeling approaches to guide preclinical animal studies and integrating PK/PD and mechanistic modeling. The upgrade of SimBiology is together with Matlab which is R2016b version now.

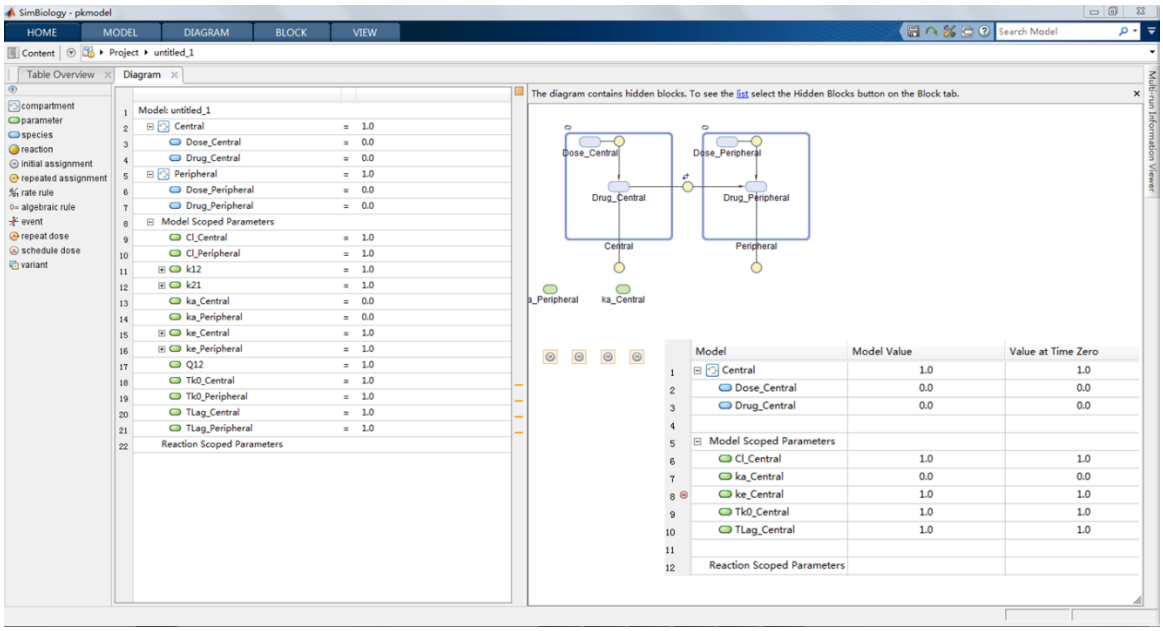


Figure 10. Interface of SimBiology

2.2 COMPARTMENT MODELS

2.2.1 One-compartment model

Compartment model is a robust tool for PK study. The simplest form is the one compartment model. This model assumes the whole body is a single system called central compartment.

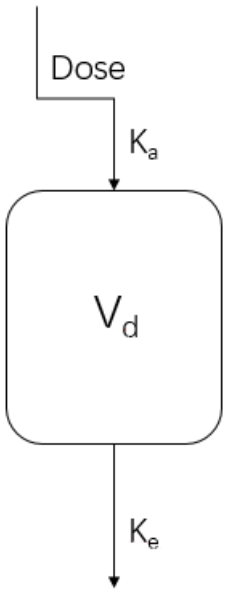


Figure 11. Diagram of one compartment model

The compartment is characterized by distribution volume V_d. As we choose the oral administration, the input depends on dose and absorption rate K_a while the output is described by elimination rate K_e. In most cases, we consider K_a, K_e as first order terms which means the relationship between ln(concentration) and time is linear.

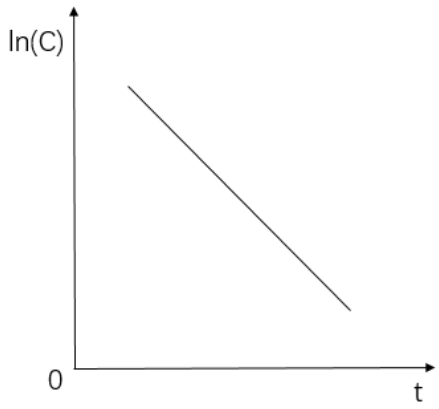


Figure 12. ln(c) versus time profile (first order)

Basic solution for concentration-time profile is:

$$\frac{dC}{dt} = -kC^n$$

C is the concentration, n is the order number, and k represents the constant.

First order means n=1, $\frac{dC}{dt} = -kC$.

While taking absorption rate into account,

$$\frac{dC}{dt} = K_a D - K_e C$$

The general denotation of initial concentration value C_0. [47]

$$C_0 = \frac{D}{V_d}$$

D is the dose, V_d is the volume of distribution.

However, for oral dose, C_0 cannot be directly used as the simple form. Thus, we use the C_{max} to replace C_0. [48]

$$C_{max} = \frac{fD}{V_d e}$$

C_{max} is the maximum concentration the drug can reach in a certain compartment (usually in plasma), F is the bioavailability, e is the base of natural logarithms.

The solution of the ubiquitous function: [49]

$$\mathrm{C} = \frac{fD}{V_d}\frac{K_a}{K_a - K_e}[\exp(-K_e t) - \exp(-K_a t)]$$

2.2.2 Two-compartment model

In this case, the body is taken as two compartments, central and peripheral. Central compartment is the site where the drug well distributes. Correspondingly, the

peripheral compartment only has limited number of drug molecules existing.

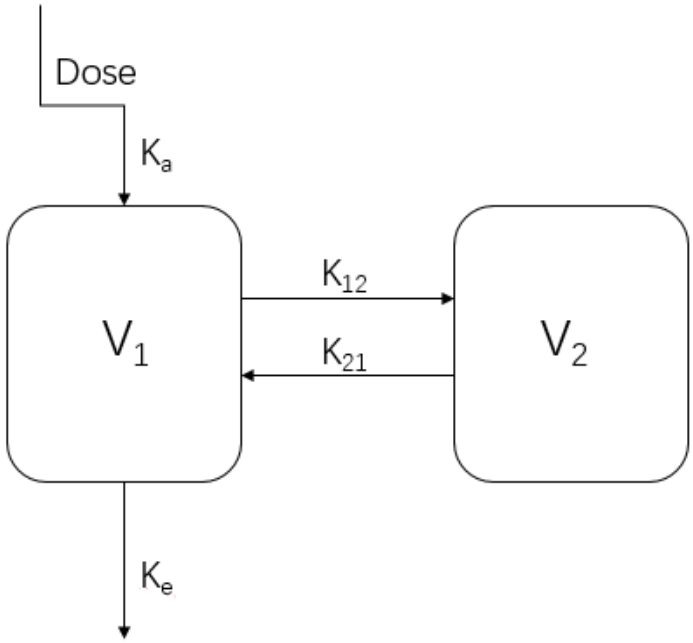


Figure 13. Diagram of two-compartment model

V_1 is the distribution volume of central compartment, V_2 is the distribution volume of peripheral compartment, K_{12} is the distribution rate, K_{21} is the redistribution rate.

We still adopt first-order elimination.

$$\frac{dC}{dt} = -kC$$

The general solution for two-compartment model concentration (i.v.). [50]

$$\mathrm{C} = \mathrm{A}e^{-\alpha t} + Be^{-\beta t}$$

The general solution for oral administration. [51]

$$\mathrm{C} = \mathrm{A}'e^{-\alpha t} + B'e^{-\beta t} + Ce^{-k_a t}$$

The solution of the ubiquitous function: [52]

$$C = K_a C_0 [\frac{(K_{21} - \alpha)}{(K_a - \alpha)(\beta - \alpha)} e^{-\alpha t} + \frac{(K_{21} - \beta)}{(K_a - \beta)(\alpha - \beta)} e^{-\beta t} + \frac{(K_{21} - K_a)}{(\alpha - K_a)(\beta - K_a)} e^{-\alpha t}]$$

Here C_0 is the absorbed concentration. Therefore, the solution should be transformed to: $C_0 = \frac{fD}{V_1}$

α,β term can be obtained through equations below: [53]

$$b = K_{12} + K_{21} + K_e$$

$$\alpha = \frac{b + \sqrt{b^2 - 4K_{21}K_e}}{2}$$

$$\beta = \frac{b - \sqrt{b^2 - 4K_{21}K_e}}{2}$$

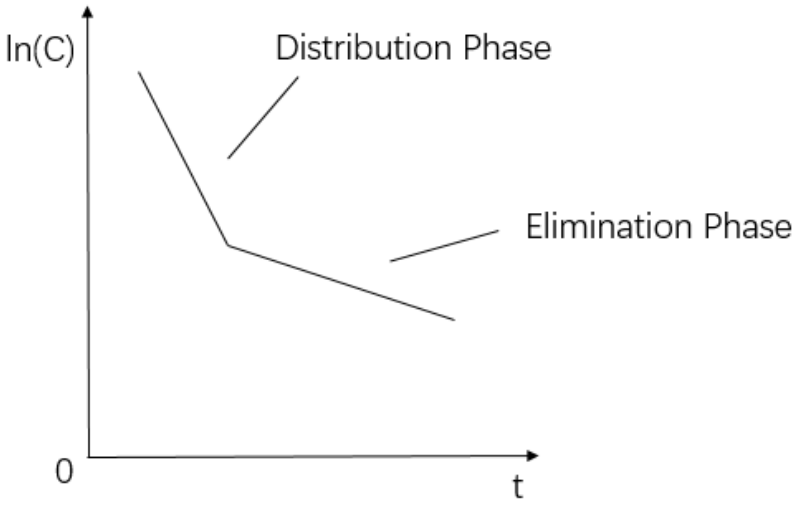


Figure 14. ln(concentration) versus time profile of two-compartment model

In the plot of concentration-time profile, the figure can be divided to 2 linear parts, distribution and elimination phase, with the slope of -α and -β respectively.

Besides one-compartment and two-compartment models, we can use any number of compartments to study pharmacokinetic problems. Generally speaking, the more compartments, the more accurately the question will be identified. However, restricted by time, labor, financial cost, researchers are not able to adopt models with enormous compartments. Also, there is no golden standard to instruct the model usage. People should choose the model according to their own conditions.

2.3 Kinetic Models & Drug-Drug Interaction

2.3.1 Metabolite formation kinetics

As this is an enzyme involved reaction, we adopted Michaelis-Menten Kinetics [54]:

$$v = \frac{V_{max} \times S}{K_m + S}$$

v represents the metabolite formation of velocity, K_m the Michaelis-Menten constant of the substrate, V_{max} the maximum formation velocity for the enzyme, and S the substrate concentration [55].

To apply the Michaelis-Menten Kinetics, we need to introduce the new formula to demonstrate this equation. As mentioned in Chapter 1.3, diazepam can be regarded as

the substrate and inhibitor of CYP3A4 which means this is an inhibition model. There are three typical types, competitive, noncompetitive and uncompetitive inhibition.

In competitive inhibition, the inhibitor competes with the substrate at the enzyme's same active site.

$$\mathrm{E} + \mathrm{S} \leftrightarrow \mathrm{ES} \rightarrow \mathrm{E} + \mathrm{P}$$

$$\mathrm{E} + \mathrm{I} \leftrightarrow \mathrm{EI}$$

E is the enzyme, S is the substrate, ES is the substrate-enzyme complex, P is the product, I is the inhibitor, EI is the enzyme-inhibitor complex [56].

The K_m of competitive inhibition could be illustrated as:

$$K_{m(inhibition)} = K_m \times (1 + \frac{I}{K_i})$$

$$\mathrm{v} = \frac{V_{max} \times S}{K_m \left(1 + \frac{I}{K_i}\right) + \mathrm{S}}$$

Ki is the dissociation constant of the inhibition-enzyme complex.

As the inhibitor only reacts with the enzyme, more substrate needs more inhibitor.

As to noncompetitive inhibition, in addition to binding with the substrate itself, the inhibitor also binds to the enzyme-substrate complex. The binding usually happens at a site remote from active site.

$$\mathrm{E} + \mathrm{S} \leftrightarrow \mathrm{ES} \rightarrow \mathrm{E} + \mathrm{P}$$

$$\mathrm{E} + \mathrm{I} \leftrightarrow \mathrm{EI}$$

$$\mathrm{EI} + \mathrm{S} \leftrightarrow \mathrm{EIS}$$

$$\mathrm{ES} + \mathrm{I} \leftrightarrow \mathrm{EIS}$$

$$V_{\max(inhibition)} = \frac{V_{max}}{(1 + \frac{I}{K_i})}$$

$$\mathrm{v} = \frac{\frac{V_{max} \times S}{(1 + \frac{I}{K_i})}}{K_m + S}$$

Noncompetitive inhibitor is not affected by the concentration of substrate. It exerts inhibition mainly through reducing the binding affinity of substrate or enzyme.

About uncompetitive model, instead of the substrate, the inhibitor only binds to the substrate-enzyme complex.

$$\mathrm{E} + \mathrm{S} \leftrightarrow \mathrm{ES} \rightarrow \mathrm{E} + \mathrm{P}$$

$$\mathrm{ES} + \mathrm{I} \leftrightarrow \mathrm{EIS}$$

$$K_{m(inhibition)} = K_m \times (1 + \frac{I}{K_i})$$

$$V_{\max(inhibition)} = \frac{V_{max}}{(1 + \frac{I}{K_i})}$$

$$\mathrm{v} = \frac{\frac{V_{max} \times S}{(1 + \frac{I}{K_i})}}{K_m(1 + \frac{I}{K_i}) + S}$$

To inhibit such reaction, we need higher concentration of substrates and enzymes.

In terms of the limited number of binding sites on CYP 3A4 and the same kind of chemical reaction these two drugs conduct (N-methylation), we speculate that this is a competitive inhibition. Oxycodone and Diazepam bind to the same CYP3A4 active site.

2.3.2 Two identical site competition model

Because Oxycodone and Diazepam both undergo phase I metabolism through CYP3A4 and N-demethylated to Noroxycodone and nordiazepam, respectively, we consider them as a two-site model with competition [57]. The scheme of this model is shown as below.

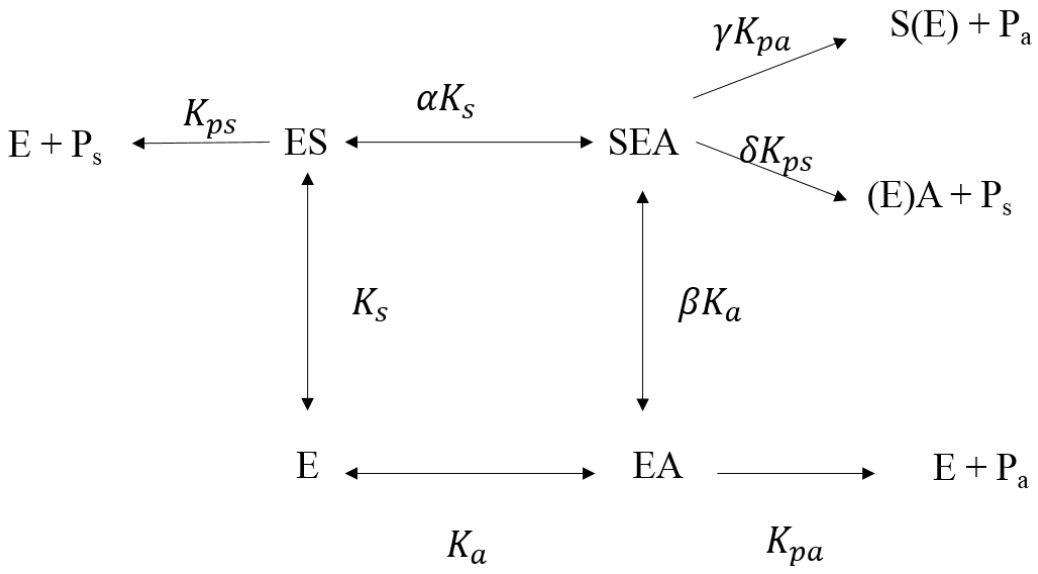


Figure 15. Scheme of two site competition model for oxycodone and diazepam

E: CYP3A4 enzyme, S: oxycodone, A: diazepam, P1: diazepam metabolite, P2: oxycodone metabolite, Ks: oxycodone binding affinity, Ka: diazepam binding affinity, Kps: catalytic rate of oxycodone, Kpa: catalytic rate of diazepam, α: the factor how diazepam affects oxycodone binding, β: the factor how oxycodone affects diazepam binding, γ: the factor how the complex SEA affects diazepam reaction, δ: the factor how the complex SEA affects oxycodone reaction. If α, β, γ, δ >1, the rate is increased, α, β, γ, δ <1, the rate is decreased.

Hill equation:

$$\frac{v}{V_{max}} = \frac{S^{nH}}{S_{0.5}^{nH} + S^{nH}}$$

$S_{0.5}$ is the substrate concentration when $v = \frac{V_{max}}{2}$, n_H is the Hill coefficient [58]. The Hill equation was introduced by A.V. Hill [59]. The equation is derived from a binding reaction scheme [60]. It is a very useful tool for ligand concentration estimation and determining the degree of cooperativity of the ligands binding to the enzyme [60].

To put the equation into this kinetic model, we change the form of Hill equation to:

$$\frac{v}{V_{max}} = \frac{\frac{S}{K_s} + \frac{S^2}{\alpha K_S^2} + \frac{\gamma \cdot \delta \cdot S \cdot A}{\alpha \cdot \beta \cdot K_S \cdot K_a}}{1 + \frac{2S}{K_S} + \frac{S^2}{\alpha K_s^2} + \frac{2SA}{\alpha \beta K_S K_a} + \frac{2A}{K_a} + \frac{A^2}{\beta K_a^2}}$$

2.3.3 Drug-drug interaction

To measure the degree of inhibition, we use the formula:

$$\mathrm{R} = \frac{v(+inhibitor)}{v(-inhibitor)} = \frac{1}{1 + \frac{I}{K_i}}$$

v(+inhibitor) is the metabolic rate with inhibition while v(-inhibitor) is without inhibition.

First, we need to determine the hepatic clearance CL_h. Three models can be used to quantify the CL_h.

Well-stirred model [61]:

$$CL_h = \frac{Q_h \cdot f_u \cdot CL_{int}}{Q_h + f_u \cdot CL_{int}}$$

Parallel tube model [61]:

$$CL_h = Q_h[1 - \exp\left(-\frac{f_u \cdot CL_{int}}{Q_h}\right)]$$

Dispersion model[61]:

$$CL_h = Q_h[1 - \frac{4a}{(1+a)^2 \exp\left[\frac{(a-1)}{2Dn}\right] - (1-a)^2 \exp\left[-\frac{(a+1)}{2Dn}\right]}]$$

$$\mathrm{a} = \sqrt{1 + 4RnDn}$$

$$\mathrm{Rn} = \frac{f_u \cdot CL_{int}}{Q_h}$$

CL_{int} is the intrinsic clearance, Q_h is the hepatic drug flow, f_u is the blood unbound fraction, Dn is the axial dispersion number, Rn is the efficiency number [62].

$$f_h = \frac{CL_h}{CL_h + CL_r}$$

$$f_m = \frac{CL_{int,1}}{CL_{int,1} + CL_{int,2}}$$

f_h is the fraction of hepatic clearance in total clearance, CL_r is the renal clearance, f_m is the fraction of the metabolic process subject to inhibition in CL_h, $CL_{int,1}$ and $CL_{int,2}$ are the intrinsic clearance for the metabolic pathway inhibited and not inhibited.

For orally administered drugs, the degree of inhibition can be expressed as:

$$R = \frac{AUC_{po}(+inhibitor)}{AUC_{po}(-inhibitor)}$$
$$= \frac{1}{f_h \cdot \frac{CL'_h}{CL_h} + 1 - f_h} \cdot \frac{F'_h}{F_h}$$

F_h is the hepatic availability.

In this way, we'll be able to predict the change of the AUC value.

To figure out the differential equations, we need the stoichiometric matrix [63].

The general form of the matrix is as follows.

$$\begin{array}{c} \\ \text{Type A} \\ \text{Type B} \\ \text{Type C} \end{array} \begin{array}{c} \begin{array}{cc} \text{Primary} & \text{Secondary} \end{array} \\ \begin{bmatrix} present & 0 \\ present & present \\ 0 & present \end{bmatrix} \end{array}$$

Primary means primary compound, secondary means secondary compound. Type A, B, C are metabolic pools. A: biochemical element conservation. B: conservation of exchanged biochemical moiety. C: cofactor conservation. [64]

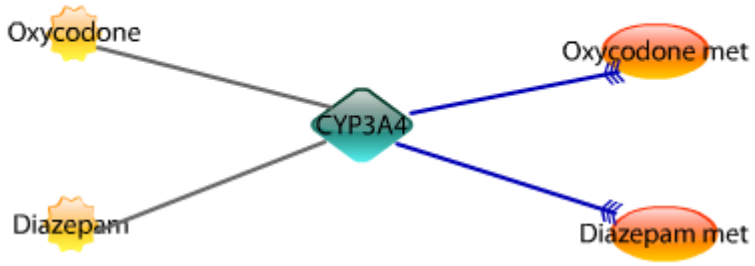


Figure 16. Reaction map of the Oxycodone and Diazepam enzymatic reactions

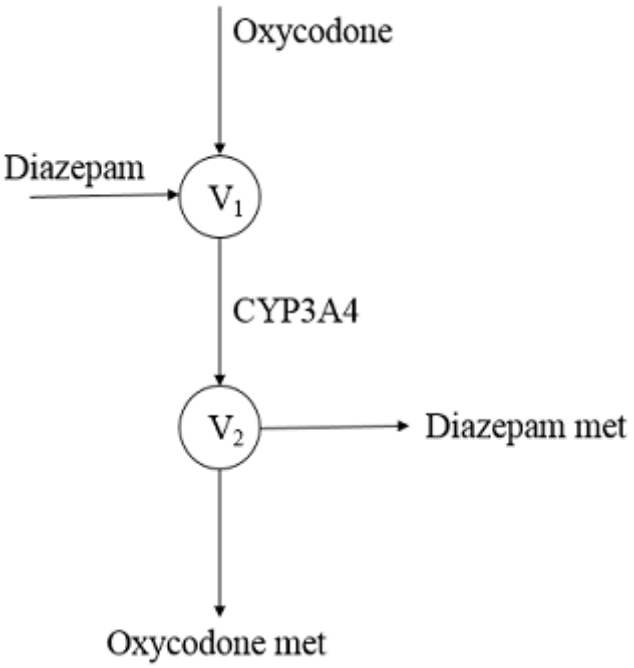


Figure 17. Compound map of the Oxycodone and Diazepam enzymatic reactions

Oxycodone: O, Diazepam: D, CYP3A4: E, Oxycodone met: OM, Diazepam met: DM.

$$\begin{matrix} O & D & E & OM & DM \end{matrix}$$
$$\begin{bmatrix} 1 & 0 & 1 & 1 & 0 \\ 0 & 1 & 1 & 0 & 1 \end{bmatrix}$$

There are several ways to describe generalized reaction rate [65].

Linear approximation:

$$\mathrm{r} = r_0 + k(e - e_0) + \sum_i k_i(c_i - c_{0,i})$$

Linear in logistic approximation:

$$\mathrm{r} = r_0 + kln\left(\frac{e}{e_0}\right) + \sum_i k_i \ln\left(\frac{c_i}{c_{0,i}}\right)$$

Mass action in S-systems:

S-systems are nonlinear approximation models using power-law formalism [66].

$$\mathrm{r} = r_0 k \frac{e}{e_0} \prod_i (\frac{c_i}{c_{0,i}})^{k_i}$$

Thermokinetic, linear-logistic models:

$$\mathrm{r} = \frac{e}{e_0}(k + \sum_i k_i \ln(\frac{c_i}{c_{0,i}}))$$

Michaelis-Menton models:

$$\mathrm{r} = \mathrm{e}(\prod_i \frac{c_i}{k_i + c_i} - K \prod_j \frac{c_j}{k_j + c_j})$$

As a result, the reaction rate formula can be summarized as:

$$r_j = r_j^{max}(e_j) f_j(c_j, p_j)$$

r : reaction rate, e : enzyme, c_i, c_j :concentration of metabolite, i, j, K: $\frac{k_{-1}}{k}$, k, k_i : kinetic parameters, r_0, e_0, c_0: initial value.

Based on all these discussed above, we can derive the differential equations.

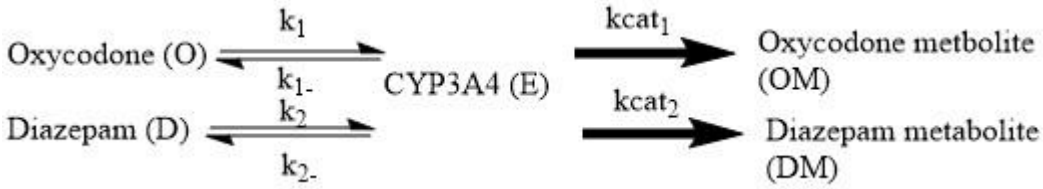


Figure 18. Simplified diagram pathway

Differential equations are as below [67].

$$\frac{dE}{dt} = k_1[O] - k_{1-}[E] + k_2[D] - k_{2-}[E] - k_{cat1}[OM] - k_{cat2}[DM]$$

$$\frac{dO}{dt} = -k_1[O] + k_{1-}[O] - k_{cat1}[OM]$$

$$\frac{dD}{dt} = -k_2[D] + k_{2-}[D] - k_{cat2}[DM]$$

$$\frac{dOM}{dt} = k_{cat1}[OM]$$
$$\frac{dDM}{dt} = k_{cat2}[DM]$$

When combined with competitive inhibition rate equation:

$$\mathrm{v} = \frac{V_{max} \times S}{K_m\left(1 + \frac{I}{K_i}\right) + \mathrm{S}}$$

$$\mathrm{v} = \frac{dOM}{dt} = k_{cat2}[DM] = \frac{V_{max} \times O}{\frac{k_{1-}}{k_1}\left(1 + \frac{D}{\frac{k_{2-}}{k_2}}\right) + \mathrm{O}}$$

3.0 RESULTS

3.1 STATISTICAL ANALYSIS

I got the data from an online database, Pharmapendium (https://www.pharmapendium.com/#/home). This database is comprised of seven data types. They are pharmacokinetic data, metabolizing enzyme & transporter data, drug safety data, FAERS (FDA Adverse Event Reporting System) data, chemistry data, efficacy data and activity data. I used the FAERS data of oxycodone hydrochloride to investigate the significance of oxycodone safety and co-administration problem. I mainly utilize the columns which are primary suspect drugs, other administered drugs and outcomes. The interface of the oxycodone hydrochloride FAERS dataset is shown in Figure 19.

	Case ID	Primary Suspect Drugs	Other Administered Drugs	Adverse Events	Outcomes	FDA Date	Gender	Age
0	3766882	Acetaminophen; Oxycodone Hydrochloride, Oxyc...	Aspirin, Bicalutamide, Ciprofloxacin Hydro...	Cardiomegaly, Dizziness, Fatigue, Heart ...	Hospitalization, LifeThreatening	Thu Feb 21 00:00:00 GMT 2002	Male	79
1	3766776	Oxycodone Hydrochloride		Acute respiratory failure, Cardiac arrest, ...	Death	Tue Feb 19 00:00:00 GMT 2002	Female	67
2	3730652	Oxycodone Hydrochloride	Alprazolam, Dromostanolone Propionate, Fen...	Bronchopneumonia, Cardiac arrest, Cardiome...	Death	Wed Feb 27 00:00:00 GMT 2002	Male	34
3	3770770	Oxycodone Hydrochloride		Death	Death	Wed Feb 27 00:00:00	Male	19

Figure 19. Interface of Pharmapendium database

This dataset includes 40996 records in FDA adverse effects reporting system for oxycodone. Among these patients, 20012 have other drugs co-administered which means about half of the patients use other medications together with oxycodone hydrochloride. 13425 patients ended up dead. More than half of the dead patients (7530) have concurrent drug use history. According to the drug list of benzodiazepines family, we define the search condition as 'Diazepam or Oxazepam or Alprazolam or Chlordiazepoxide or Clorazepate or Estazolam or Flurazepam or Temazepam or Triazolam. As a result, 4926 patients have used benzodiazepines with oxycodone hydrochloride. Among them, 2668 people died which means the death rate is 54.2%, higher than the overall statistical death rate, 32.7%. As to the benzodiazepines involved in death-outcome patients, 43.6% are diazepam. In addition, we calculate the odds ratio (OR) of benzodiazepines as:

$$\mathrm{OR} = \frac{ad}{bc} = \frac{2668 \times 25313}{2258 \times 10757} = 2.78$$

a, b, c, d is defined as: [68]

a= Number of exposed cases

b= Number of exposed non-cases

c= Number of unexposed cases

d=Number of unexposed non-cases

Here, for our OR value, the exposure status refers to benzodiazepines involved while the outcome status means death.

In summary, about half of the Oxycodone hydrochloride users have other drugs administered which aggravates the fatal consequences compared with oxycodone

single use. The statistical result also fits the fact that the use of opioids with benzodiazepines is very common.

Table 1 Statistical results of Pharmapendium FAERS data

Total number of data: 40996
Number of patients who have other drugs administered: 20012 (48.8%)
Number of patients whose outcome contains Death: 13425 (Overall Death Rate: 32.7%)
Number of dead patients who have other drugs administered: 7530 (56.1% of dead patients)
Number of patients who have benzodiazepines administered: 4926 (24.6% of other drugs)
Number of dead patients who have benzodiazepines administered: 2668 (benzodiazepines death rate: 54.2%)
Number of patients who have diazepam administered: 2148 (43.6% of benzodiazepines involved)
Number of died patients who have diazepam administered: 1164 (diazepam death rate: 54.19%)

3.2 SINGLE OXYCODONE ADMINISTRATION

To lay the foundation of DDI, we perform the single drug simulation to quantify the parameters and validate the model.

3.2.1 Single dose simulation

By searching the literature online, we will be able to determine the parameter range and validate the model. Currently, we choose the one-compartment model to perform

the simulation.

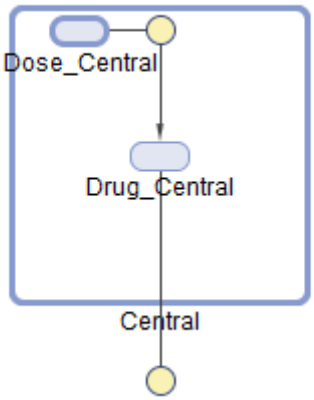
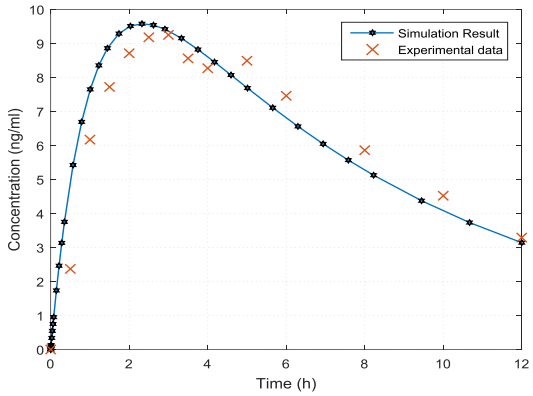


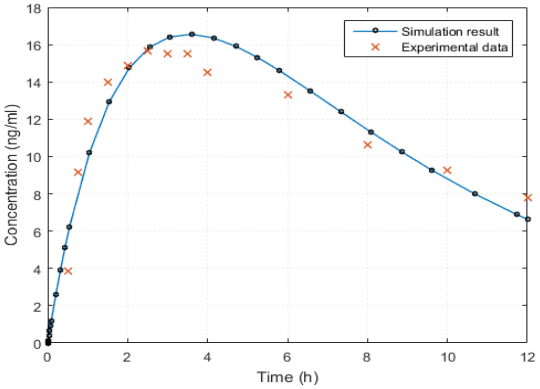
Figure 20. Diagram of one-compartment model on Simbiology

As shown, we create a one-compartment model with bolus dosing and linear clearance elimination. According to a literature [69], K_a value is set between 0.13 to 0.18 hour^{-1}. K_e can be derived from half-life ($t_{1/2}$). For the first-order elimination, $K_e = \frac{0.693}{t_{1/2}}$ [70]. The Oxycodone half-life is about 3 to 5 hours. The K_e should be estimated in the range of 0.1386 to 0.231 hour^{-1}.

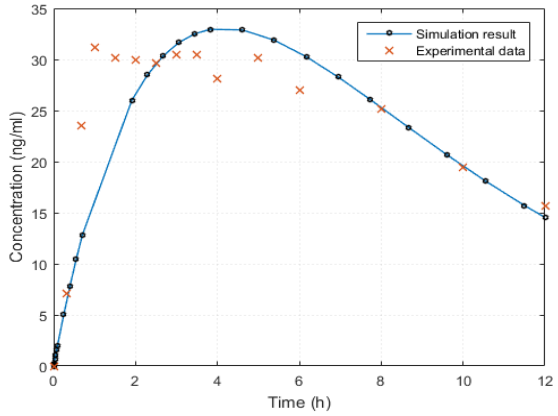
We extract the experiment data from the report of the pharmaceutical company, Blenheim Pharmacal, Inc. They tested the concentration-time profile under different dose of Oxycodone. By administering 10, 20, 40, 80, 160 mg single dose of Oxycodone, they attained the concentration versus time plot.


Simulation Result
Experimental data
Concentration (ng/ml)
Time (h)

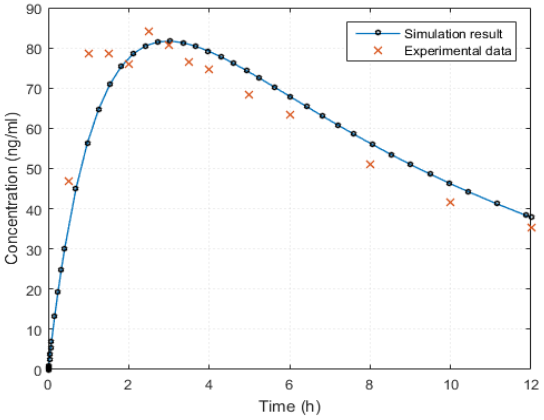
(a)


Simulation result
Experimental data
Concentration (ng/ml)
Time (h)

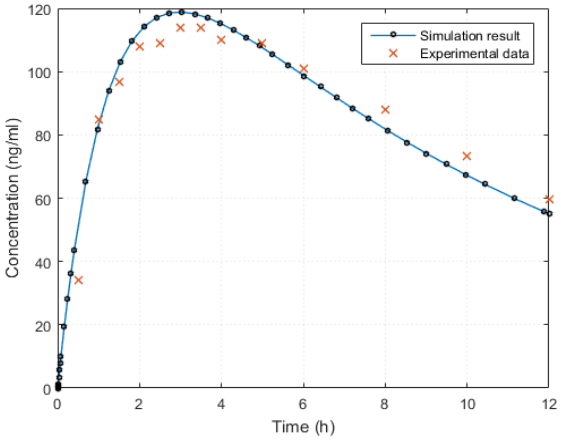
(b)


Simulation result
Experimental data
Concentration (ng/ml)
Time (h)

(c)


Simulation result
Experimental data
Concentration (ng/ml)
Time (h)

(d)


Simulation result
Experimental data
Concentration (ng/ml)
Time (h)

(e)

Figure 21. Time course of Oxycodone fitting plot under different doses (a) 10mg dose (b) 20 mg dose (c) 40 mg dose (d) 80 mg dose (e) 160 mg dose

In comparison to the experimental data, the simulation results can fit well. We can ensure the parameters as:

Table 2. K_a, K_e parameter values

Dose/mg	10	20	40	80	160
K_a/h^{-1}	0.13	0.11	0.11	0.14	0.11
K_e/h^{-1}	0.2166	0.1952	0.1868	0.1824	0.1733

The table shows these values fall in the estimated range. This proves the feasibility to use the one-compartment model as the preliminary tool for our PK study

3.2.2 Multi-dose simulation

In real-life medication, the drug is supposed to be given in specific dose several times a day to maintain the drug concentration at a steady level. The concentration solution for multi oral dose should be: [69]

$$(C_P)_{t(single)} = \frac{fD}{V}\frac{K_a}{K_a - K_e}[\exp(-K_e t) - \exp(-K_a t)]$$

$$(C_p)_{t(multiple)} = (C_p)_{t1} + (C_p)_{t2} + \cdots\cdots (C_p)_{tn+1}$$

A paper described the Oxycodone dosing problem and provided information about multi-dose Oxycodone concentration-time profile [71]. Single dose administration can reach a relatively high concentration but will be eliminated soon and lose the pharmacological efficacy. The multi-dose simulation of Oxycodone is as below.

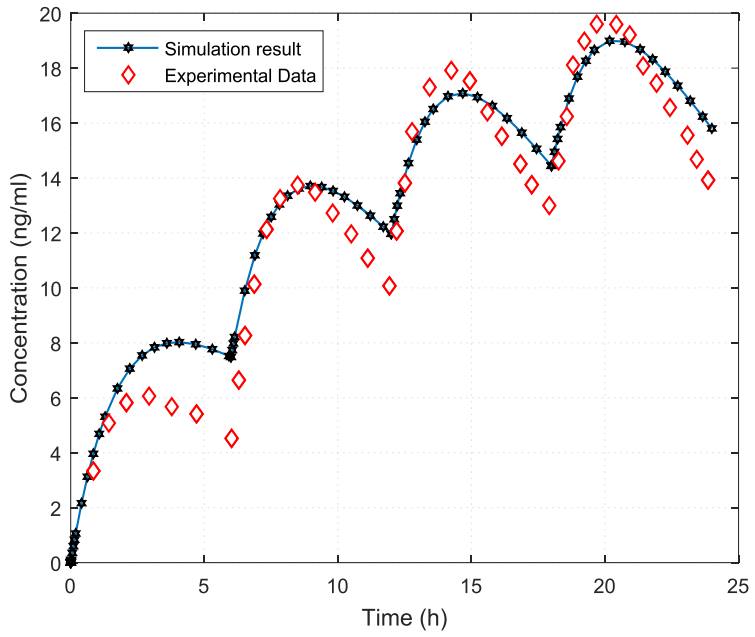


Figure 22. Time course of multi-dose Oxycodone 5 mg / 6 h, 24 h fitting plot

During the 24 h time range, patients were given Oxycodone in 5 mg dose for 4 times (every six hours). We can see that after the first-time administration, the C_{max} was up to 6 ng/ml as single dose. Until the third administration, the drug level keeps in a narrow range, between 14 ng/ml and 20 ng/ml. In the simulation, we still chose the parameters from that value range in Table 2. The simulation result corresponds with

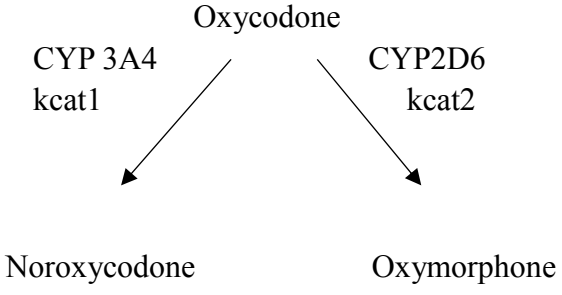
experimental data.

3.3 DDI SIMULATION

To simulate the DDI between Oxycodone and Diazepam, we introduce a PK/PD model. There are two one-compartment models in the diagram. One represents Oxycodone as well as the other indicates Diazepam. In order to integrate these two drugs, we brought in a PD model.

3.3.1 The rough prediction of the DDI effects

Our proposed mechanism is the Diazepam's inhibition to Oxycodone metabolism as, they bind to CYP3A4 competitively. Michaelis-Menten kinetics is used to mechanistically characterize their metabolism. The formation of N, O-demethylation metabolic products, Noroxycodone and Oxymorphine occurs in liver microsomes [72]. When it comes to Oxycodone metabolism, we try to simulate the profiles of the main metabolites, Noroxycodone and Oxymorphone.



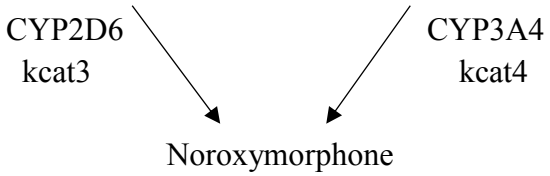


Figure 23. Simplified Oxycodone metabolic pathway

The reaction rates and parameter values are:

k_bind1= [kf1, kr1]

kcat1 =[kf1, kr1, kc1]

k_bind2= [kf2, kr2]

kcat2= [kf2, kr2, kc2]

k_bind3= [kf3, kr3]

kcat3= [kf2, kr3, kc3]

k_bind4= [kf4, kr4]

kcat4= [kf4, kr4, kc4]

kf1=1e-5, kr1= 0.05, kc=1e-1

kf2=1e-5, kr2=1e-1, kc=1e-1

kcat2= kcat3= kcat4

Each catalytic reaction is defined as the combination of reverse binding rate and catalytic rate. The result is calculated by ODEs.

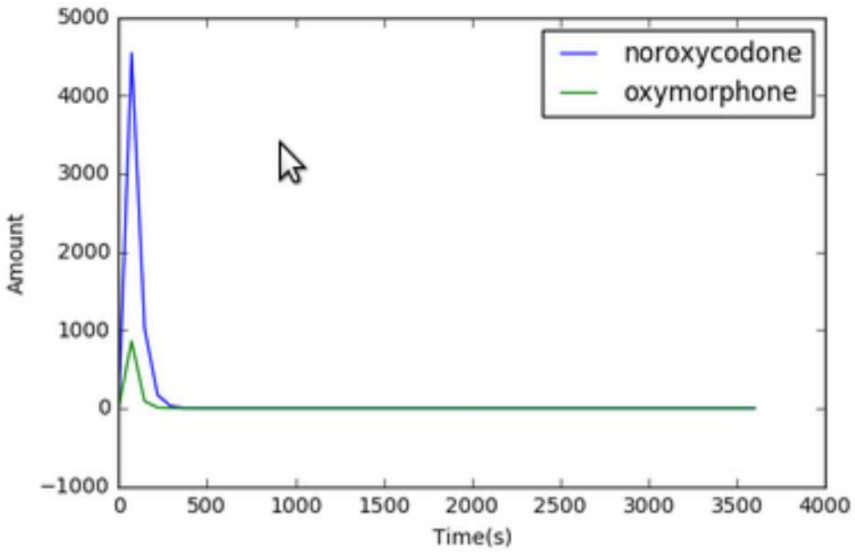


Figure 24. Time course of metabolites of Oxycodone

The ratio of the two metabolites, Noroxycodone and Oxymorphone, is 4:1 which is in consistent with Figure 22. As is well known, the existence of Diazepam will undoubtedly alter the Oxycodone metabolism. Thus, in the next step, we will first keep the rate value the same as the single Oxycodone simulation and see the change on the major metabolite, Noroxycodone.

We simplify the process as:

$$\text{Oxycodone} \xrightarrow[k_{bind1}]{\text{CYP 3A4}} \text{Noroxycodone} \xrightarrow[k_{cat1}]{\text{CYP 2D6}} \text{Noroxymorphone}$$

$$\text{Diazepam} \xrightarrow[k_{bind2}\ k_c]{} \text{Oxyzepam} \xrightarrow[k_{cat2}]{\text{UGT}} \text{UGT Products}$$

Figure 25. Simplified DDI involved metabolic pathway

We define the reaction rate as:

k_bind1= [kf1, kr1]

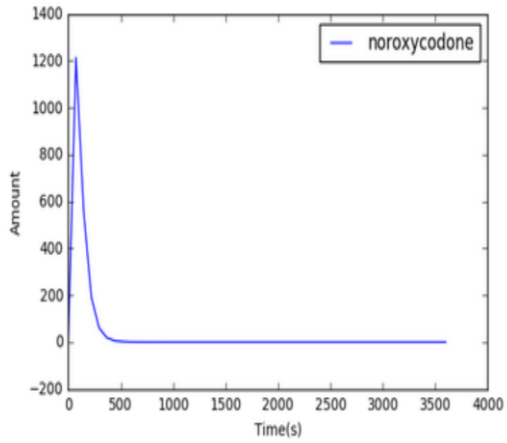
k_bind2= [kf2, kr2]

k_bin3= [kf3, kr3]

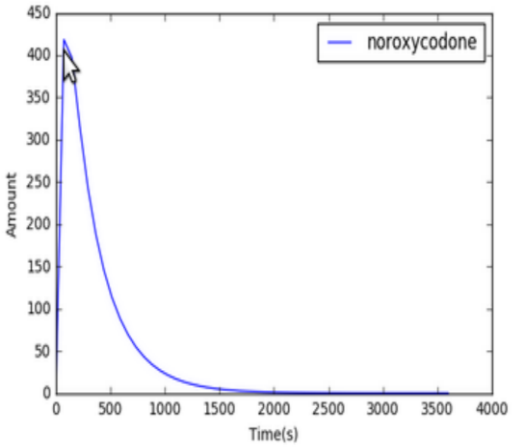
k_bind4= [kf4, kr4]

kcat1= [kf3, kr3, kc1]

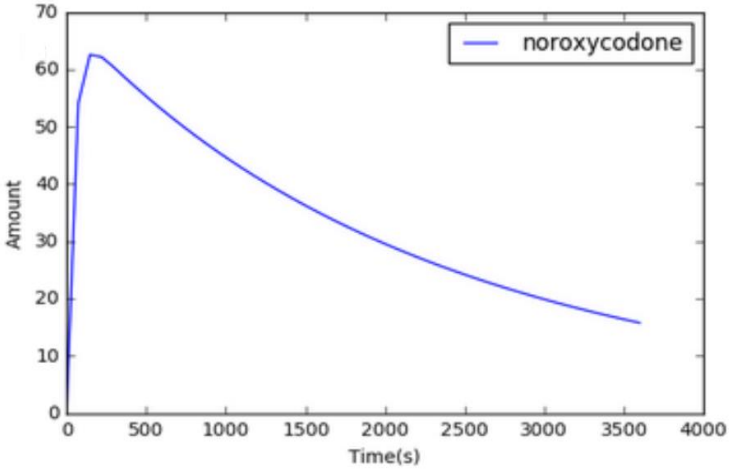
kcat2= [kf4, kr4, kc2]



(a)



(b)



(c)

Figure 26. Time Course of major metabolite noroxycodone under co-administration (a) kf1=1e-5 (b) kf1=1e-6 (c) kf1=1e-7

By comparing Figure 22 and Figure 23. (a), we can see although each parameter remains unchanged, the amount of metabolite is significantly reduced in co-administration. On account of the competitive inhibition between Oxycodone and Diazepam binding with CYP3A4, the binding rate will definitely vary. As is shown in Figure 23. (b) and (c), if the oxycodone binding rates are decreased to 1e-6 and 1e-7, the amounts of Oxycodone metabolites are decreased as well. Furthermore, the retention time of Noroxycodone is prolonged which proves that diazepam causes the delay of oxycodone metabolism. Such results roughly verify our conjectures.

3.3.2 PK/PD modeling for DDI simulation

To simulate this concomitant use of Oxycodone and Diazepam, we use a PD model to integrate the interaction between these two drugs.

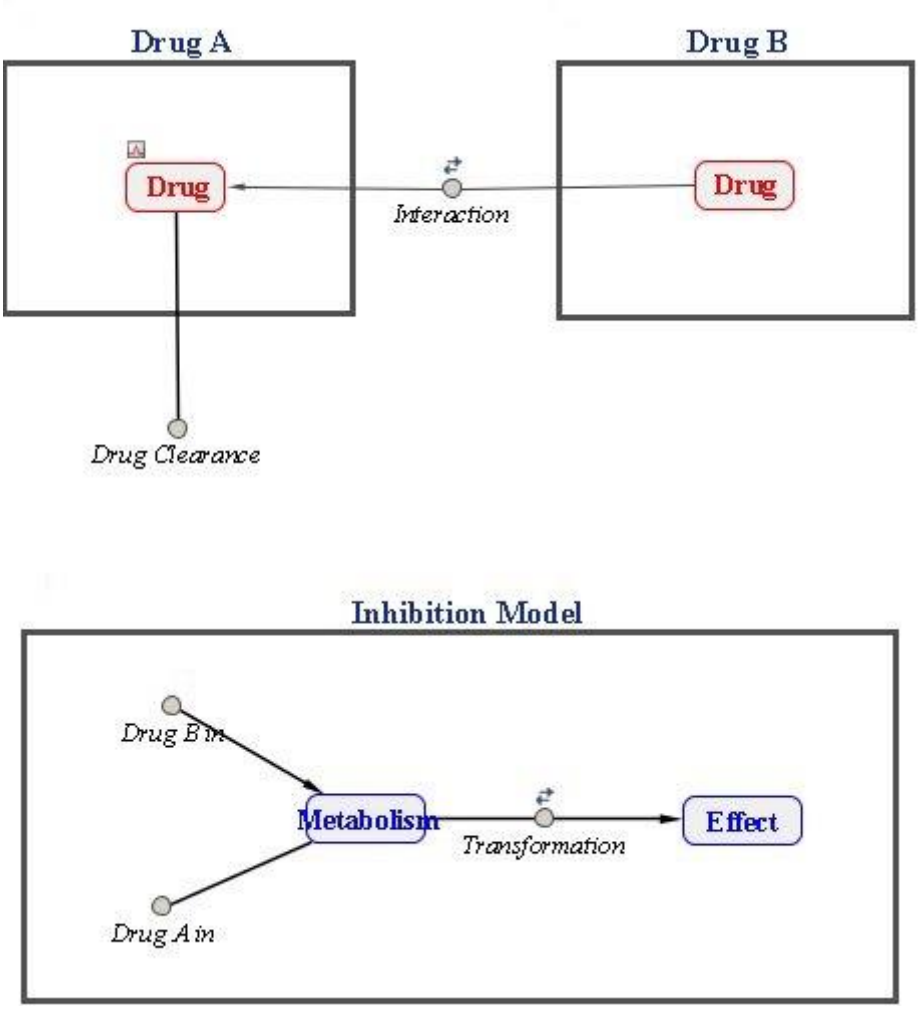


Figure 27. Diagram of DDI

Drug A represents Oxycodone, Drug B is Diazepam. On the basis of drug labeling for normal patients (e.g. not for patients with withdrawal syndromes), the Oxycodone dose often ranges from 10 to 40 mg while the dose of Diazepam is 2, 5 or 10 mg. The interaction term characterizes Diazepam's influence on Oxycodone clearance. In the reaction terms 'Drug A in' and Drug B in', we introduce the Drug A and Drug B as one of the species. We presume the drug goes through the metabolism and then

produce pharmacological effect. We define the expression at the form of Michaelis-Menten kinetic equations and use a parameter fu to denote the competitive inhibition. The Km, Vmax values can be gained from the literature [73].

We select the combination of 40 mg Oxycodone + 5 mg Diazepam to perform the simulation. The concentration-time profile is shown as below.

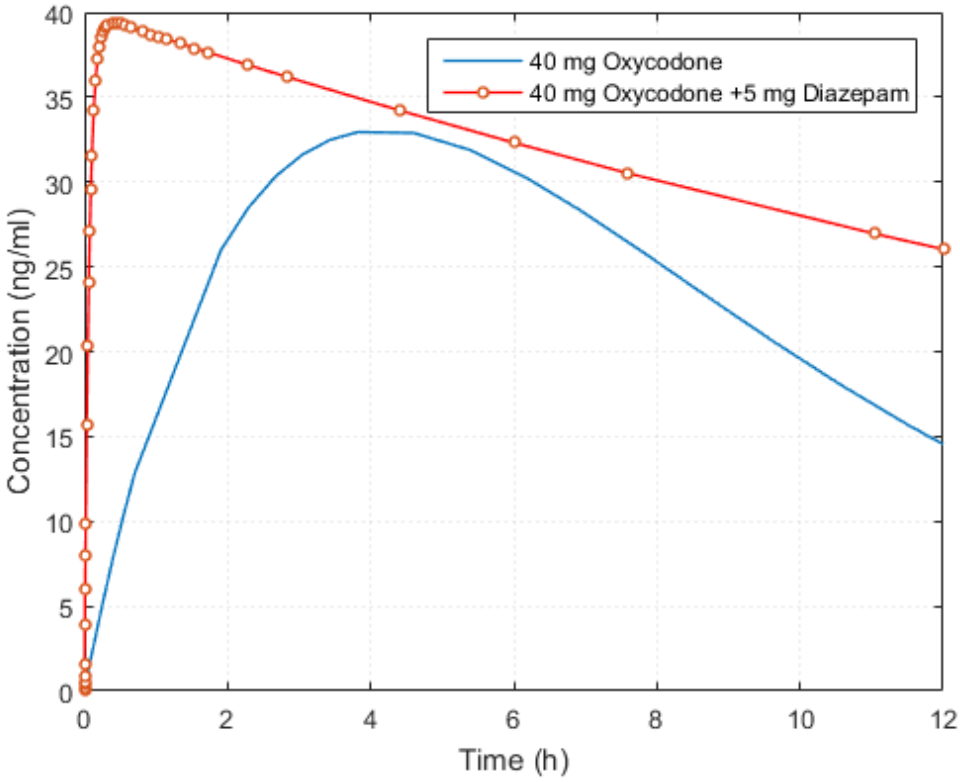


Figure 28. Simulated concentration-time profile of 40 mg single dose Oxycodone & 40 mg Oxycodone + 5 mg Diazepam

Figure 28. illustrates under the same time range 12 h, the Oxycodone concentration in co-administration is higher than that of single use. Using the inhibition degree R to characterize this co-administration,

$$R = \frac{AUC_{po}(+inhibitor)}{AUC_{po}(-inhibitor)} = \frac{388.1532}{295.0164} = 1.3157$$

This result is consistent with the prediction of major metabolite, Noroxycodone in section 3.3.1. As the concurrent use of Diazepam, the primary compound Oxycodone exists in the plasma at a higher level and longer time while the metabolite amount is comparatively low, shown in Figure 28. This study explains why the co-administration of Oxycodone and Diazepam, in other words, prescription opioids and benzodiazepines renders more threatening overdose morbidity and mortality.

4.0 CONCLUSION & FUTURE PERSPECTIVE

4.1 CONCLUSION

Our statistical result indicated the significance of this co-administration problem which exacerbates the adverse effects and increases the mortality. We have built a one-compartment model to simulate the Oxycodone concentration-time profile and validate parameter values. By constructing the co-administration model, we could obtain the Oxycodone concentration-time profile under concurrent use and verify our conjecture about the elevated AUC value. Such results allow us to draw the conclusion as while co-administered with Diazepam, the metabolism of Oxycodone is delayed which maintains the drug concentration at a relatively high level and renders more threatening overdose symptoms.

Our research provides an option to quantitatively describe the drug concomitant use. We propose to apply the results for further investigation and real-life drug use guidance.

4.2 FUTURE PERSPECTIVE

Currently, we have only adopted a one-compartment model to conduct the simulation, focusing on the plasma concentration. In future studies, we will build multi-compartment models to characterize more detailed drug-drug interactions or any other type of co-administrations.

Neural problems are very complex and hard to fully investigate. There may be other causes leading to this aggravated overdose liability. In this study, we got the evidence from FDA drug labeling and other related literature. Reported overdose symptoms and side effects provide us the rationale for speculation. In this Oxycodone case, although the major mechanism of action indicates no overlapped pathways or targets, we cannot rule out the possibilities. We will further explore potential mechanisms of drug-drug interactions with our established computational chemogenomics technologies.

So far, we have obtained all sorts of data from online resources such as databases, literature and case reports. Because Oxycodone and Diazepam are pervasively used drugs with a comparatively long history, we are able to attain the data. However, data are not available for all the potentially abused drugs and their combinations. To strictly validate the models and results, we still need to perform the experiments. We are supposed to collect the data from hospital emergency departments and incubate the liver microsomes to imitate the metabolic pathway to assess the drug-abuse-related co-administration.

Finally, we intend to expand the scope of prediction. Our lab has constructed a chemogenomics database for drug abuse research. On the foundation of this specific DDI study, we propose to establish a platform for versatile drug abuse research based on the quantitative simulations. For example, users, like patients or physicians, input the drug names and will then receive all sorts of relevant information. Furthermore, this database can evolve into a DDI prediction tool. As long as the users are aware of

drug names, the platform can provide the safety information to guide use of each drug. The aim of our project is to identify the threat of drug-abuse-related co-administration, thus to better assess toxicity and improve decision making.

APPENDIX A. ABBREVIATION

DDI	Drug-drug interaction
PK	Pharmacokinetics
PD	Pharmacodynamics
CNS	Central nervous system
CYP	Cytochrome P450
FAERS	FDA adverse event reporting system
ODE	Ordinary differential equation
ADME	Administration, distribution, metabolism, elimination
PBPK/PD	Physiologically based pharmacokinetic and pharmacodynamic
MBDD	Model based drug development
CL	Clearance
AUC	Area under the curve
OR	Odds ratio

APPENDIX B. CORE CODES

```
#import data and function
import pandas as pd
import numpy as np
data=pd.read_csv('C:/Users/ZHZ85/test/FAERS.csv',usecols=
[1,2,3,4,5,6,7,8])
#print the data
data
#print the name of each column
data.columns

#extract the Outcomes column
data1=data.Outcomes
#find the cells which contain Death. if so, print True;
else,print False
data2=data1.str.contains('Death',na=False)
#count the number of True and False outcomes
data2.value_counts()
#extract the Other Administered Drugs column
data3=data.iloc[:,2]
#convert the data from series to array
data4=np.array(data3)
#restrict the format of blank cells and replace them by 0
a=data3[1]
for i in data4:
```

```
    if i ==a:
        data5=data3.replace(i,0)
data6=np.array(data5)
#count the number of nonzero values in the array
cnt=np.count_nonzero(data6)
#print the number of patients who have other drugs
administered
Cnt

data7=np.array(data2)
# find the place of nonzero values
indice=np.where(data6!=0)
#set the values of corresponding place: if this place is True,
set the value as 1; else, set the value as 0. Calculate the
sum value of the array.
for j in indice:
    data8=data7[j]
for i in data8:
    if i=='True':
        i=1
    else:
        i=0
#print the number of died patients who have other drugs
co-administered
print np.sum(data8)

#filter the cells that contain benzodiazepines
data9=data3.str.contains('Diazepam|Oxazepam|Alprazolam|Ch
lordiazepoxide|Clorazepate|Estazolam|Flurazepam|Temazepam
|Triazolam')
```

```
#replace those cells with True, else, with False
data9.value_counts()
data10=np.array(data9)
data11=data10.astype(int)
indice2=np.where(data11==1)
for j in indice2:
    data12=data7[j]
for i in data12:
    if i=='True':
        i=1
    else:
        i=0
#print the number of dead patients who have benzodiazepines
administered
print np.sum(data12)

#filter the cells that contain benzodiazepines
data13=data3.str.contains('Diazepam')
data13.value_counts()
data14=np.array(data13)
data15=data14.astype(int)
indice3=np.where(data15==1)
for j in indice3:
    data14=data7[j]
for i in data14:
    if i=='True':
        i=1
    else:
        i=0
```

```
#print the number of dead patients who have diazepam
administered
print np.sum(data14)

# obtain the number of dead patients who do not have
benzodiazepines
data9.value_counts()
indice4=np.where(data11==0)
for i in indice4:
    data16=data7[i]
for i in data16:
    if i=='True':
        i=1
    else:
        i=0
print np.sum(data16)
```

```
#import functions
from pysb import *
from pysb.macros import catalyze
from pysb.macros import catalyze_one_step
from pysb.macros import bind
from pysb.integrate import odesolve
from matplotlib import pyplot as plt
import numpy as np

# define all the below codes into a model
Model()

#set the parameter values
kf1= 1e-5
kr1= 0.05
kc1 = 1e-1
klist1 =[kf1, kr1]
klist_cat1 = [kf1,kr1,kc1]
kf2= 1e-5
kr2= 1e-1
kc2 = 1e-1
klist2 =[kf2, kr2]
klist_cat2 = [kf2,kr2,kc2]

#set the initial values
Parameter('CYP3A4_0',10000)
Parameter('oxycodone_0',20000)
Parameter('CYP2D6_0',6000)
```

```
# define all the mentioned substance as monomer form
Monomer('oxycodone',['a','b'])
Monomer('CYP3A4',['a','d'])
Monomer('CYP2D6',['b','c'])
Monomer('noroxycodone',['c'])
Monomer('oxymorphone',['d'])
Monomer('noroxymorphone',['e'])

# set the primary compound as initial
Initial(CYP3A4(a=None,d=None),CYP3A4_0)
Initial(oxycodone(a=None,b=None),oxycodone_0)
Initial(CYP2D6(b=None,c=None),CYP2D6_0)

# define the reactions involved in oxycodone metabolic pathway
catalyze(CYP3A4(),'a',oxycodone(),'a',noroxycodone(c=None),klist_cat1
)
catalyze(CYP2D6(),'b',oxycodone(),'b',oxymorphone(d=None),klist_cat2)
catalyze(CYP2D6(),'c',noroxycodone(),'c',noroxymorphone(e=None),klist
_cat2)
catalyze(CYP3A4(),'d',oxymorphone(),'d',noroxymorphone(e=None),klist_
cat2)

# set the output compound as observable
Observable('onor',noroxycodone(c=None))
Observable('ooxy',oxymorphone(d=None))

# calculate the ODE
t= np.linspace(0, 3600)
out = odesolve(model, t)
```

```
# plot
plt.plot(t,out['onor'],label='noroxycodone')
plt.plot(t,out['ooxy'],label='oxymorphone')
plt.xlabel("Time(s)")
plt.ylabel("Amount")
plt.legend(loc='best')
plt.show()


#import functions
from pysb import *
from pysb.macros import catalyze
from pysb.macros import catalyze_one_step
from pysb.macros import bind
from pysb.integrate import odesolve
from matplotlib import pyplot as plt
import numpy as np
# define the whole thing as a model
Model()

#set the parameter values
kf_bind1 = 1e-7
kr_bind1 = 0.15
kcat = 1e-1
kf_bind2=1e-5
kr_bind2=1e-1
klist_bind1=[kf_bind1, kr_bind1]
klist_bind2=[kf_bind2, kr_bind2]
```

```
klist_cat1 = [kf_bind1,kr_bind1,kcat]
klist_cat2 = [kf_bind2,kr_bind2,kcat]

#set the initial values
Parameter('CYP3A4_0',10000)
Parameter('oxycodone_0',20000)
Parameter('CYP2D6_0',6000)
Parameter('diazepam_0',5000)
Parameter('UGT_0',5000)
Parameter('kc',1e-1)

# define all the mentioned substance as monomer form
Monomer('oxycodone',['a'])
Monomer('diazepam',['b'])
Monomer('CYP3A4',['a','b'])
Monomer('noroxycodone',['c'])
Monomer('CYP2D6',['c'])
Monomer('noroxymorphone',['e'])
Monomer('oxyzepam',['d'])
Monomer('UGT',['d'])
Monomer('ugtp',['f'])
# set the primary compound as initial
Initial(oxycodone(a=None),oxycodone_0)
Initial(diazepam(b=None),diazepam_0)
Initial(CYP3A4(a=None,b=None),CYP3A4_0)
Initial(CYP2D6(c=None),CYP2D6_0)
Initial(UGT(d=None),UGT_0)
```

```
# define the binding and catalytic reactions during Oxycodone and Diazepam co-administration
bind(CYP3A4(),'a',oxycodone(),'a',klist_bind1)
bind(CYP3A4(),'b',diazepam(),'b',klist_bind2)
Rule('CYP3A4_catalyze',CYP3A4(a=1,b=2)%oxycodone(a=1)%diazepam(b=2)>>
CYP3A4(a=None,b=None)+oxyzepam(d=None)+noroxycodone(c=None),kc)
catalyze(CYP2D6(),'c',noroxycodone(),'c',noroxymorphone(e=None),klist_cat2)
catalyze(UGT(),'d',oxyzepam(),'d',ugtp(f=None),klist_cat2)

# set Noroxycodone as the output
Observable('onor',noroxycodone(c=None))

# calculate the ODE
t= np.linspace(0, 3600)
out = odesolve(model, t)

# plot
plt.plot(t,out['onor'],label='noroxycodone')
plt.xlabel("Time(s)")
plt.ylabel("Amount")
plt.legend(loc='best')
plt.show()
```

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